Remarks/Arguments

Reconsideration of the above-identified application in view of the present amendment is respectfully requested.

By the present amendment claims 1-4 and 7-21 have been cancelled. New claims 25-44 have been added to the application. New claims 25-44 include similar limitations as previous claims 1-4 and 7-21, but have been rewritten to clarify the claimed subject matter. New claims 25-44 are limited to antibodies so claims 25-44 fall within the elected claims set. Below is a discussion of the 35 U.S.C. 112 first paragraph rejection. All references cited in this amendment are included with this amendment as attachments.

Claims 1-4 and 7-21 were rejected under 35 U.S.C. 112 as failing to comply with the enablement requirement. The Office Action argues as a first matter that Factor B is a component of the alternate complement pathway and that one in the art would not see any method that is specifically dependent upon inhibition of Factor Ba as having relevance in the control of the classical pathway. The Office Action adds that the specification discloses various in vitro assays that show anti-Factor B antibodies are capable of binding to Factor B and of inhibiting the complement cascade via the alternative complement pathway, but that the specification fails to show the treatment of any condition by using anti-Factor B antibodies or any other reagent. The Office Action notes that there is no correlation between any disease "resulting from" activation of the alternative complement pathway and the claimed method of treatment.

As noted above, claim 1-4 and 7-21 have been cancelled. New claim 25 recites a method of inhibiting factor B-dependent complement activation in blood of a subject in need thereof. The method comprises administering to blood of the subject an amount of an anti-factor B antibody effective to inhibit complement activation, the anti-factor B antibody in an in vitro assay preventing factor B binding to C3b and properdin-bound C3b; for preventing the formation of Bb; reducing C3a, *C5a,* and C5b-9 generation; reducing C3 conversion into C3a and C3b; reducing *C5* conversion into C5a and C5b; reducing the activation of neutrophils, monocytes and platelets; and/or inactivating cells bearing C3a and C5a receptors.

Claim 25 is enabled by the present application because one having ordinary skill in the art would not require undue experimentation to practice the claimed invention. With respect to the Office Action's first point that one in the art would not see any method that is specifically dependent upon inhibition of Factor B as having relevance in the control of the classical pathway, the applicant respectfully disagree that one skilled in the art would not see the relationship between inhibition of factor B function and control of the classical pathway.

As noted in the background of the specification, the state of the prior art at the time of filing the present application teaches that:

> "The alternative pathway C3 convertase is stabilized by C3b bound properdin. Since the substrate for the alternative pathway C3 convertase is C3, C3 is therefore both a component and a product of the reaction. As the C3 convertase generates increasing amounts of C3b, an amplification loop is established. Furthermore, the classical pathway can also generate C3b. This newly generated C3b can bind factor B and thereby engage the alternative pathway for the propogation of the classical pathway. This allows more C3b to

deposit on the target. Both the classical and alternative pathways converge at C3, which is cleaved to form C3b and C3a. C3a is a potent anaphylatoxin and has been implicated in the pathogenesis of a variety of clinical indications. C3a activates neutrophils, monocytes, platelets, mastcells, and T lymphocytes. C3a has been shown to be important for the induction of paw edema in an adjuvant-induced arthritis model. Addition of C3b to C3 convertase generates C5 convertase, which cleaves C5 to produce C5b and C5a. C5a is the most potent anaphylatoxin that causes alterations in smooth muscle, in vascular tone, and in vascular permeability. It is also a powerful chemotaxin and an activator of neutrophils, monocytes, platelets, endothelial cells, and T lymphocytes. C5a-mediated cellular activation can significantly amplify inflammatory responses by inducing the release of additional inflammatory mediators, including cytokines, hydrolytic enzymes, arachadonic acid metabolites and reactive oxygen species."

Thus, regardless of the trigger agent, the classical (and the lectin) pathway utilize the amplification loop of the alternative pathway. The C3 convertases of all three pathways cleave C3 into C3b, which can participate with factor B in forming additional alternative pathway C3 convertase (C3bBb). The alternative pathway C3 convertase is stabilized by the binding of properdin. Properdin extends the alternative pathway C3 convertase half-life six to ten fold. Addition of C3b to the C3 convertase leads to the formation of the alternative pathway C5 convertase. Cleavage of C5 by C5 convertases generates C5b and C5a.

C3b is therefore the integral part of the C5 convertase of the classical pathway, and of C3/C5 convertases of the alternative pathway. The C3 convertase of the classical pathway (C4bC2a) cleaves C3 into C3b and C3a. The C3b molecule generated as a result of classical pathway activation can bind properdin and factor B of the alternative pathway to generate the alternative pathway C3a and C5

convertases. As a result, the amplification loop takes off and starts generating additional C3b molecule thus providing amplification and perpetuation of the classical pathway via this amplification loop. Using inhibitors of the alternative pathway specific proteins, factors P and D, the total contribution of the amplification loop in classical pathway has been evaluated by two groups. (Mol Immunol, 2000. 37(5): p. 191-201**).** In a classical pathway mediated event, blockade of the alternative pathway proteins, factors P and D using blocking antibodies, have shown that greater than 80-90% of the total classical pathway activation was downregulated. These data clearly suggest a substantial role of the alternative pathway amplification loop in classical pathway activation. These data are further supported by studies using factor B knock out mice which demonstrated lower classical pathway hemolytic activity in factor B deficient mice compared to wild type controls, thus reflecting the loss of alternative pathway amplification loop and its role in contributing to classical pathway functions.

The alternative pathway, in addition to its widely accepted role as an independent pathway for complement activation, also provides an amplification loop for complement activation that is initially triggered via the classical and lectin pathways (Schweinle, J. E., et al., J. Clin. Invest. 84:1821-1829, 1989). In this alternative pathway-mediated amplification mechanism, C3 convertase (C4b2b) generated by activation of either the classical or lectin complement cascades cleaves C3 into C3a and C3b, and thereby provides C3b that can participate in forming C3bBb, the alternative pathway C3 convertase.

Accordingly, based on the specification of the present application and the state of the art at the time of filing the patent application, one skilled in the art would readily

see inhibition of Factor B as having relevance in the control of the classical pathway and complement activation in general.

Claim 33 is enabled because it includes similar limitations as claim 25 and therefore one having ordinary skill in the art would not require undue experimentation to practice the invention recited in claim 33.

Claims 32, 39, and 40 recite diseases in which complement activation is associated. The Office Action as noted above argues that there is no correlation between any disease "resulting from" activation of the alternative complement pathway and the claimed method of treatment. The above noted claims have been amended to recite that complement activation is associated with these diseases. Claims 32, 39, and 40 are enabled because the state of the art at the time of the application was such that one skilled in the art in view of the present application would recognize that complement activation is associated with such claimed diseases.

Despite its essential role in immune defense, the complement system contributes to tissue damage in many clinical conditions. For example, with respect to extracorporeal circulation (ECC), blood is exposed to foreign surfaces that alters normal hemostasis and often leads to activation of neutrophils, monocytes, and platelets. In cardiopulmonary bypass (CPB) surgery, blood is routed through the Heart-and-Lung machine where patient's blood comes in contact with surfaces of the long tubings, oxygenator, and filters. Ultimately a systemic inflammatory reaction sets in that can cause significant inflammatory responses.

The CPB-triggered inflammatory reaction can result in post-surgical complications, generally termed "postperfusion syndrome." Included under which are cognitive deficits, respiratory failure, bleeding disorders, renal dysfunction and, in the most severe cases, multiple organ failure. Patients who have an exaggerated inflammatory response to CPB tend to bleed more, require more respiratory support, demonstrate greater capillary leakage via weight gain, and display a decline in independent functioning relative to normal responders. Thus, it appears that the magnitude of the inflammatory response to CPB adversely influences clinical outcomes (Inflamm Res, 2002. 51(12): p. 579-86). Coronary bypass surgery with CPB (on pump) leads to profound activation of complement compared to surgery without CPB (off pump). A number of studies evaluated complement activation byproducts such as C3a, C5a, and sC5b-9 and nearly all such studies suggested significant elevation in concentration of such products (Ann Thorac Surg. 1994 Mar; 57(3):781-3).

Both C3a and C5a are potent stimulators of neutrophils, monocytes, and platelets (Circulation, 1999. 100(5): p. 553-8, J Clin Invest, 1995. 96(3): p. 1564-72). MAC also has been shown to activate neutrophil and platelets during ECC (J Clin Invest, 1995. 96(3): p. 1564-72, Complement, 1986. 3(3): p. 152-65). Activation of these cells results in release of proinflammatory cytokines (IL-1, IL-6, IL-8, TNF alpha) (Scand J Clin Lab Invest, 1995. 55(1): p. 79-86) oxidative free radicals and proteases. C5a has been shown to upregulate expression of the adhesion molecules, CD11b and CD18 of Mac-1 in polymorphonuclear cells (PMNs) and to induce degranulation of PMNs to release proinflammatory enzymes. C5b-9 induces the expression of the adhesion molecule, P-selectin (CD62P), on platelets, whereas both C5a and C5b-9

induce surface expression of P-selectin on endothelial cells. These adhesion molecules are involved in cell to cell interaction among leukocytes, platelets and endothelial cells. The expression of adhesion molecules on activated endothelial cells is responsible for sequestration of activated leukocytes at sites of inflammation. These cells then mediate tissue inflammation and injury. It is the actions of these complement activation products on neutrophils, monocytes, platelets and other circulatory cells that likely lead to the various problems that arise after CPB. It is the alternative complement pathway that is activated in cardiopulmonary bypass based on concrete evidence provided by the presence of the split products of factor B, Ba (Eur J Cardiothorac Surg, 1990. 4(6): p. 291-6).

As a result of ECC, several acute and chronic complications occur which could cause potentially life-threatening medical problems. Many of these complications occur due to activation of the complement system, which then plays an important role in the development of acute and chronic inflammation that leads to the many symptoms of postperfusion syndrome. These inflammatory events are primarily mediated via activated neutrophils, monocytes and platelets. The complement activation byproducts, C3a, C5a, and sC5b-9 (MAC), are known to activate all three cell types via C3a and C5a receptors or by direct insertion of MAC into the cellular bilayer. Both, C3a and C5a receptors have been found on neutrophils, monocytes, and T lymphocytes. Activation of proinflammatory cells causes elevated levels of elastase, peroxides, and a whole range of cytokines including TNF alpha and IL-1 beta that contribute to the inflammatory response. Activated platelets can become dysfunctional depending upon the potency of stimulus. Complement-mediated platelet

dysfunction can result in both excessive thrombosis and excessive bleeding as platelets first become activated and then become spent and non-functional, and are removed from the circulation.

ECC clearly causes complement activation, as demonstrated by the sequence of inflammatory events that are known to occur when cardiac patients go for bypass procedures. In such inflammatory conditions, elevated levels of C3a, C5a, and MAC have been found in plasma. In addition, activated neutrophils, monocytes, and platelets are found in blood following CPB. Inflammatory byproducts of activated inflammatory cells are also found in the plasma, these include; TNF, elastase, and peroxides. The established tubing loop model of CPB was originally established by Gong et al (J Clin Immunol, 1996. 16(4): p. 222-9) and later modified by Gupta-Bansal (Mol Immunol, 2000. 37(5): p. 191-201) to evaluate complement and cellular activation markers of inflammation following the rotation of blood in a loop of tubing. The tubing loop model of CPB uses method that are similar to those used in the clinic during bypass where blood is diluted with plasmalyte and subjected to the ECC model of CBP. As a result of blood contact coming in contact with the artificial surface of the tubing, both complement and cellular activation of neutrophils, monocytes and platelets occurs.

In the complement system there are at least 25 complement proteins. Complement components achieve their immune defensive functions by interacting in a cascading series of intricate but precise enzymatic cleavage and membrane binding events. The complement cascade progresses via the classical pathway, lectin pathway or the alternative pathway. These pathways share many components, and

while they differ in their initial steps, they converge and share the same "terminal complement" components responsible for the activation, attack, and destruction of target cells. The classical complement pathway is typically initiated by antigen-antibody complexes, while the alternative pathway is usually antibody independent, and can be initiated by bacterial, pathogen, or artificial surfaces.

The lectin pathway is considered to be a variation of the alternative pathway. All three pathways require the amplification loop of the alternative pathway for propogation and augmentation of the pathway. Both classical and alternative pathways converge at complement component C3, which upon cleavage releases C3a and C3b. C3a is a potent anaphylotoxin and is resposnsible for complex inflammatory effects. C3a activates monocytes (Circulation, 1999. 100(5): p. 553-8.), lymphocytes (Photochem Photobiol, 2006. 82(2): p. 558-62, Nature, 2000. 406(6799): p. 998-1001), promotes arthritis (Arthritis Rheum, 2002. 46(4): p. 934-45), causes the release of IL-6 and IL-8, and is known to cause cognitive impairment (Eur J Cardiothorac Surg, 2003. 23(3): p. 334-40, Br J Anaesth, 2000. 84(3): p. 378-93, Neuropsychology, 2002. 16(3): p. 411-21, Ann Thorac Surg, 2003. 75(2): p. S715-20, Ann Thorac Surg, 1996. 61(5): p. 1342-7, N Engl J Med, 2001. 344: p. 395-402, Ann Neurol, 2005. 57(5): p. 615-21, Neurol Clin, 2006. 24(1): p. 133-45). C3a has also been implicated in urticaria, rhinitis and asthma (J Thorac Cardiovasc Surg, 1999. 118(3): p. 460-6). The C3a/C5a receptors are widely expressed in neurons and astrocytes and may modulate neuronal function (Arthritis Rheum, 2002. 46(4): p. 934-45). C5a is another anaphylatoxin that is involved in induction of arthritis (J Exp Med, 2002. 196(11): p. 1461-71) brain

inflammation (Glia, 2002. 38(2): p. 169-73), and asthma (Am J Respir Crit Care Med, 2001. 164(10 Pt 1): p. 1841-3).

Both C3a and C5a cause platelet aggregation (Naunyn Schmiedebergs Arch Pharmacol, 1976. 295(1): p. 71-6). The anaphylatoxins, C3a and C5a, which are released with activation of complement components, trigger mast cell degranulation, which releases histamine and other mediators of inflammation that results in early events that set the stage for inflammation, such as smooth muscle contraction, increased vascular permeability, leukocyte activation, and other inflammatory phenomena. C5a also functions as a chemotactic peptide that serves to attract pro-inflammatory granulocytes to the site of complement activation. In addition, direct activation of endothelial cells by C5a induces the release of the coagulation-inhibiting glycoprotein heparan sulfate.

The cleavage product of C5, C5b, combines with C6, C7, and C8 to form the C5b-9 complex at the surface of the target cell. After a sufficient number of MACs insert into target cell membranes, the openings (MAC pores) these MACs create mediate rapid osmotic lysis of the target cells.

Additionally, anaphylatoxins are known to activate neutrophils by upregulating the expression of CD11b during bypass. As a result, neutrophils adhere to the endothelium of blood vessels and start cytotoxic events. In CPB, elevation in CD11b levels have been reported (European Journal of Cardio-Thoracic Surgery, Vol 10, 279-283, Copyright © 1996 by European Association for Cardio-thoracic Surgery, Patterns of changes in neutrophil adhesion molecules during normothermic cardiopulmonary bypass. A clinical study F Le Deist, P Menasche, A Bel, J Lariviere, A Piwnica and G

Bloch) which initiates endothelium and renal tissue damage by releasing toxic substances. CD11b on neutrophils is also associated with respiratory burst and myosite injury (1: J Clin Invest. 1992 Oct;90(4):1335-45, Neutrophil induced oxidative injury of cardiac myocytes. A compartmented system requiring CD11b/CD18-ICAM-1 adherence. (Entman ML, Youker K, Shoji T, Kukielka G, Shappell SB, Taylor AA, Smith CW.). Activated neutrophils, upon degranulation, release elastase, lactoferin, and myeloperoxidase, substances which clearly play roles in acute lung injury in patients undergoing bypass. Elastase secretion has been shown to help in neutrophil transmigration through the endothelial barrier. In contrast to neutrophils, where the predominant inflammatory response is initiated by the C5a and C5b-9 molecules of complement, monocyte activation and activation of CD11b is primarily regulated by C3a in CPB (Circulation, 1999. 100(5): p. 553-8). Platelets bear both C3a and C5a receptors that respond to individual stimuli in guinea pig blood (J Immunol, 1981. 126(4): p. 1506-9, Naunyn Schmiedebergs Arch Pharmacol, 1976. 295(1): p. 71-6).

In an extracorporeal setting, anti-factor P, C5, and D monoclonal antibodies have been used to downregulate platelet activation in the tubing loop model of CPB (J Thorac Cardiovasc Surg, 2001. 122(1): p. 113-22, Blood. 1998 Sep 1;92(5):1661-7, Mol Immunol, 2000. 37(5): p. 191-201). Such monoclonal antibodies and a small molecule complement inhibitor also have been used in a baboon model of CPB with similar results. Both models, with and without the animal attached, demonstrate activation of neutrophils, monocytes, and platelets as a result of complement activation (Ann Thorac Surg, 2002. 74(2): p. 355-62; discussion 362).

Platelet dysfunction can also occur when platelets come in contact with the non-biological surfaces of the ECC associated with CPB. Additional mechanisms, for example, mechanical trauma due to shear stress, surface adherence, and turbulence within the extracorporeal oxygenator, may cause fragmentation of platelet membranes. Activated complement components, however, are arguably the most important factors contributing to the platelet dysfunction that are observed in associated with ECC. Within minutes after initiating CPB, bleeding time is prolonged significantly and platelet aggregation is impaired due to platelet dysfunction. These changes in bleeding time are independent of platelet count and worsen as CPB progresses. Bleeding times, which are normally less than 10 minutes, can approach 30 minutes after 2 hours of CPB.

Activated platelets express CD62P as an activation marker. This protein is a specific integral membrane protein also known as GMP-140. Several reports suggest that the expression of P-selectin, an activation marker of monocytes and neutrophils, is directly associated with platelet dysfunction, and occurs during ECC. Effects of ECC on platelets are particularly significant because platelets can only be activated once, i.e., activation of platelets decreases the number of functional platelets available when platelet functions are subsequently required. The importance of the effects of ECC on platelets is demonstrated by the finding that the impaired hemostasis observed after cardiac operations is mainly attributable to platelet dysfunction (J Thorac Cardiovasc Surg, 1988. 96(4): p. 530-4).

The activation marker, CD62P, is known to mediate the binding of platelets to leukocytes, thus causing the formation of platelet-neutrophil and platelet-monocyte

conjugates. One result of such conjugate formation is the removal of platelets from the circulation, a phenomenon that can contribute to the development of thrombocytopenia. Such leukocyte-platelet adhesion caused by P-selectin has been found to be induced by CPB (Blood, 1992. 79(5): p. 1201-5).

CPB effects on neutrophil and monocytes activation have been described in relation to the expression of the activation marker for these cell, CD11b (Blood, 1992. 79(5): p. 1201-5). Upregulation in the activation of neutrophils and monocytes is particularly relevant to CPB-induced injury since expression of CD11b/CD18 is responsible for leukocyte adherence to and penetration (diapedesis) through the endothelium via binding to the intercellular adhesion molecules ICAM-1 and ICAM-3 on "activated" endothelium. Administration of blocking CD11b antibodies have been shown to prevent reperfusion injury (J Clin Invest, 1988. 81(2): p. 624-9). In addition, increased CD11b expression on leukocytes has been linked to complications associated with hemodialysis (Blood, 1990. 75(5): p. 1037-50). Thus, based on these findings, CD11b/CD18 may contribute to ECC associated medical problems. Platelets also form conjugates with monocytes. Platelet-monocyte conjugates have been reported to occur during hemodialysis (Platelets, 1998. 9(3-4): p. 261-4) and have been identified as markers of myocardial infarction (J Am Coll Cardiol, 2001. 38(4): p. 1002-6, J Am Coll Cardiol, 1998. 31(2): p. 352-8). In addition, there is strong evidence that suggests monocyte activation by C3a induces monocytes to produce and release of proinflammatory mediators sucha as interleukin 1beta and TNF-alpha. C3a(C3adesArg) induces production and release of interleukin 1 by cultured human monocytes (J Immunol, 1987. 139(3): p. 794-9), Cytokine production during

hemodialysis: effects of dialytic membrane and complement activation (Am J Nephrol, 1996. 16(4): p. 293-9). A new biologic role for C3a and C3a desArg: regulation of TNF-alpha and IL-1 beta synthesis. Thus, collectively, the elevated levels of C3a, C5a, and MAC are responsible for cellular activation which in turn causes tissue and cellular damages seen in post perfusion syndrome complications of CPB or ECC. Activated monocytes and neutrophils also accumulate in the pulmonary vessels and vascular beds, as has been demonstrated by serial biopsies of lung tissue before and after CPB and contribute to postoperative dysfunction of the lungs (Arch Surg, 1988. 123(12): p. 1496-501). Liver, brain and pancreas, also suffer such damage, which can result in postoperative dysfunction of these organs.

The tubing loop model of CPB, which is described in the patent application, has been widely used to assess the effect of complement inhibitory drugs in the extra corporeal model. The model mimics many features of the CPB machine, but without the attached patient. Complement and cellular activation products are elevated in samples after whole blood samples are rotated in the tubing loop, these include significantly elevated levels of C3a, C5a, sC5b-9, neutrophil elastase, and TNF. Cellular analysis of blood aliquots after rotation in the tubing loop also demonstrates elevated levels of CD11b positive neutrophils and monocytes, and CD62P positive platelets. Further, analyses of serum samples indicate elevated levels of neutrophil elastase and TNF.

Published literature supports the use of simple models of extracorporeal circuits that mimic procedures such as CPB (J Clin Immunol, 1996. 16(4): p. 222-9, Immunopharmacology, 1997. **38**(1-2): p. 119-27), dialysis, plasmapheresis in an effort

to determine the efficacy of drugs to inhibit complications of such procedures. The model consists of tubing partially filled with blood and rotated at 37 ºC to allow complement activation to occur at the blood polymer and the blood air interface, conditions of CPB. Blood circulation through artificial tubing surface of the clinical CPB or ECC circuit is a foreign surface that causes complement activation. A published study evaluated the potential of a soluble recombinant form of human complement receptor type 1 to reduce complement activation associated with CPB (Ann Thorac Surg, 1993. 55(3): p. 619-24). sCR1 acts by blocking the conversion of complement component C3 into the activated components, C3a and C3b. Unfortunately, key measures of complement and platelet activation were not evaluated in this study, and neutrophil and other physiological endpoint results reported were disappointing. In addition, compstatin blocks C3. The blockade of C3 cleavage by compstatin prevents C3a and C3b production via both pathways in the tubing loop model. An inhibitor of C5 cleavage (an anti C5 monoclonal antibody) also has been evaluated in the tubing loop ECC. In this study, the anti C5 monoclonal antibody inhibited C5a and MAC formation in the ECC. Factor D inhibitors have also been tested in the tubing loop model of CPB (J Thorac Cardiovasc Surg, 2001. 122(1): p. 113-22), and have shown efficacy in this model. These inhibitory molecules when tested in the ECC model in animals, demonstrated benefits in the cardiac injury model.

With respect to ischemia reperfusion injury, this type of injury is most commonly found to be associated with alternative complement pathway activation. Ischemia reperfusion (I/R) injury occurs when a blood vessel is occluded naturally or by injury for an extended period of time, and the blood flow is restored after an extended period

of ischemia. Blood re-perfusion in an ischemic tissue causes alternative pathway activation. This situation is found associated with aortic aneurysm repair, CPB, vascular re-anastomosis in connection with organ transplants (e.g., heart, lung, liver, kidney) and digit/extremity replantation, stroke, myocardial infarction and hemodynamic resuscitation following shock and/or surgical procedures. There are at least two major factors contributing to the ischemic insult: complement activation and cellular (neutrophil, monocyte, and platelet) activation.

Kidney I/R is an important cause of acute renal failure. The complement system appears to be essentially involved in renal I/R injury. In renal I/R, animal studies with C4 knockout mice demonstrated no significant tissue protection, while C3-, C5-, C6-, and factor B knockout mice were protected from injury, suggesting that complement activation during renal I/R injury occurs via the alternative pathway (Life Sci, 2003. 74(5): p. 543-52, J Clin Invest, 2000. 105(10): p. 1363-71). Thurman et al. (J Immunol, 2003. 170(3): p. 1517-23), found that factor B-deficient mice developed no renal injury after I/R. Further, the data also suggested that wild-type mice had an increased complement C3 deposition and neutrophil infiltration after I/R, whereas factor B deficient mice demonstrated virtually no C3 deposition or neutrophil infiltration. These results further confirmed that complement activation in the kidney after I/R occurs exclusively via the alternative pathway, and that selective inhibition of this pathway could provide protection to the kidneys from ischemic insult. Using factor D deficient mice, Stahl et al. recently presented evidence for an important role of the alternative pathway in intestinal I/R in mice (Am J Pathol, 2003. 162(2): p. 449-55). In a porcine model of myocardial I/R, animals treated with monoclonal antibody ("MoAb")

to the anaphylatoxin, C5a, prior to reperfusion showed attenuated infarction. Rats treated with C5 MoAb demonstrated attenuated infarct size, neutrophil infiltration and apoptosis in the myocardium. These experimental results highlight the importance of complement activation in the pathogenesis of I/R injury. In acute myocardial infarction (AMII), the plasma levels of Bb, and SC5b-9 increased only in patients with AMI. The plasma SC5b-9 level was related to peak creatine phosphokinase ($r = 0.71$) and inversely related to the ejection fraction ($r = -0.71$). The plasma SC5b-9 level of patients with congestive heart failure was higher than that of patients without congestive heart failure in AMI (Circulation, 1990. 81(1): p. 156-63). These data further support a role for alternative pathway activation in AMI. Further evidence for a role of alternative complement pathway activation in myocardial infarction comes from the studies of Langlois et al (Atherosclerosis, 1988. 70(1-2): p. 95-105). This group evaluated the C3bBbP complexes and sC5b-9 in sera following acute MI. and concluded that complement is activated during myocardial infarction.

Reperfusion of the ischemic myocardium during acute MI is required to prevent tissue damage, however, it is associated with downstream pathology that occurs after reperfusion. This so-called "reperfusion injury" is accompanied by a marked inflammatory reaction, which contributes to tissue injury. Oxygen free radicals and activation of neutrophils and monocytes in addition to activation of complement system represents the major contributors of the inflammatory reaction upon reperfusion. In fact, activation of the complement system may induce the activation of neutrophils and monocytes and generation of oxygen free radicals associated with reperfusion injury. As a result of complement activation, C3a, C5a and the membrane attack complex

(MAC) are generated. C3a is known to activate monocytes, and C5a is known to activate neutrophils and both help attract neutrophils to the site of inflammation, leading to superoxide production. MAC is deposited over endothelial cells and smooth vessel cells, leading to tissue injury. An inhibitory drug that prevents C3a, C5a, and MAC production is expected to downregulate MI driven tissue injury.

With respect to stroke, animal studies have implicated the complement system in cerebral ischemia/reperfusion injury and suggest that complement inhibition may improve stroke outcomes. To assess the applicability of these findings to humans, a study evaluated the characteristics and time course of human complement activation after stroke, both C3a and C5a were acutely elevated after human ischemic stroke.

Additional studies also report that brain tissue damage after stroke is mediated partly by inflammation induced by ischaemia-reperfusion injury where the complement system plays a pivotal role. In neuronal ischemia, C3a and C5a were elevated compared to controls suggesting that activation of complement plays a role in neuronal ischemia (Glia, 2002. 38(2): p. 169-73). Direct evidence for role of complement comes from studies utilizing a soluble human recombinant complement receptor (sCR1) which inhibits the activation of both the classical and the alternative pathways of complement. The inhibitor prevented neutrophil accumulation into the injury site in traumatic brain injury (J Cereb Blood Flow Metab, 1995. 15(5): p. 860-4).

Complement activation plays a role in contributing to inflammation within the injured brain (Brain Res Brain Res Rev, 1998. 27(3): p. 243-56). A role for complement activation in traumatic brain injury also was shown in C3 and C5 deficient mice. C3 and C5 deficiency resulted in decreased neutrophil infiltration and reduced

injury size after a traumatic brain injury. It has been shown that debris from injured neurons or myelin breakdown products trigger complement activation, including formation of C5b-9. Activated complement components may stimulate accumulation of inflammatory cells and formation of brain edema, as well as having membrane destructive effects by the end product, MAC, thereby being mediators in the development of secondary brain damage (J Neurotrauma, 2001. 18(12): p. 1295-311).

The complement pathways also play a role in the pathogenesis of acute brain injury which involves cerebral ischemia and trauma and chronic neurodegeneration such as Alzheimer's Disease (Ann N Y Acad Sci, 2003. 992: p. 56-71). In animal model studies, complement C3 deficient mice demonstrated less brain edema and inflammatory response compared to C3 sufficient mice suggesting a role for complement in brain injury. One study evaluated the role of complement activation on cellular brain injury in patients undergoing coronary artery bypass grafting. The level of S100 beta, a marker of tissue injury, was found to be higher in patients with higher complement activation. These data suggested that activation of complement activation is related to neuronal injury.

With respect to anaphylactic shock, reports have shown that anaphylaxis can be induced in isolated guinea pig hearts in the presence of complement. In these studies anaphylaxis was due to anaphylatoxin formation given that selected depletion of C3, which causes depletion of C3a and C5a formation, prevented anaphylaxis. Further, reconstitution with C3 and C5, or even only C3, restored anaphylaxis; in fact, the greater the C3 content at the time of antigen challenge, the more intense the anaphylactic crisis (Circ Res, 1989. 65(3): p. 847-57).

With respect to atherosclerosis, complement is extensively activated in atherosclerotic lesions especially in ruptured plaques. Components of the terminal complement pathway are frequently found in human atherosclerotic plaques. The extent of C5b-9 deposition was found to correlate with the severity of the lesion. Local complement activation may induce cell lysis and generate at least some of the cell debris found in the necrotic core of advanced lesions. Sublytic complement activation could be a significant factor contributing to monocyte infiltration into the arterial intima during atherogenesis (Arterioscler Thromb Vasc Biol, 1996. 16(5): p. 673-7). Persistent activation of complement may be detrimental because it may trigger and sustain inflammation. Complement inhibition by genetic C6 deficiency also has been shown to suppress the development of atherosclerosis without affecting serum cholesterol levels.

With respect to other vascular diseases and conditions, there is evidence that complement activation contributes to the pathogenesis of many forms of vasculitis, including: Henoch-Schonlein purpura nephritis, systemic lupus erythematosus-associated vasculitis, vasculitis associated with rheumatoid arthritis (also called malignant rheumatoid arthritis), immune complex vasculitis, and Takayasu's disease. Henoch-Schonlein purpura nephritis is a form of systemic vasculitis of the small vessels with immune pathogenesis, in which activation of complement is recognized as an important mechanism.

Complement activation also plays a role in dilated cardiomyopathy. Dilated cardiomyopathy is a syndrome characterized by cardiac enlargement and impaired systolic function of the heart. Recent data suggests that ongoing inflammation in the

myocardium may contribute to the development of disease. C5b-9, the terminal membrane attack complex of complement, is known to significantly correlate with myocardial expression of TNF-alpha. In myocardial biopsies from 28 patients with dilated cardiomyopathy, myocardial accumulation of C5b-9 was demonstrated, suggesting that chronic immunoglobulin-mediated complement activation in the myocardium may contribute in part to the progression of dilated cardiomyopathy.

Additionally, following angioplasty, inflammatory mechanisms play a major role in the development of restenosis. The complement activation at the site is known to release C3a and C5a. The component, C5a, has strong chemotactic and proinflammatory effects. Enhanced complement activation prior to PTA, as measured by higher levels of C5a, was significantly associated with restenosis after SFA balloon angioplasty.

With respect to pulmonary conditions, complement plays a role in the pathogenesis of lung inflammatory disorders, such as acute respiratory distress syndrome (ARDS) (N Engl J Med, 2000. 342(18): p. 1334-49), ischemia/reperfusion acute lung injury, chronic obstructive pulmonary disease (COPD), asthma, Wegener's granulomatosis and antiglomerular basement membrane disease (Goodpasture's disease). All complement components can be produced locally in the lung by type II alveolar cells, alveolar macrophages and lung fibroblasts. Thus the complement cascade can self perpetuate within lung and lead to lung injury.

Patients with ARDS almost universally show evidence of extensive complement activation (increased plasma levels of complement components C3a and C5a), and the degree of complement activation has been correlated with the development and

outcome of ARDS. In animal models, systemic activation of complement leads to acute lung injury with histopathology similar to that seen in human ARDS. In rat models, sCR1 has a protective effect in complement- and neutrophil-mediated lung injury.

Asthma also is an inflammatory disease. Although asthma appears to be multifactorial in origin, the fact that the complement system is highly activated in the human asthmatic lung is well documented. Many features of bronchial asthma, such as smooth muscle contraction, mucus secretion and recruitment of inflammatory cells, are consistent with the activation of complement because of elevated levels of C3a and C5a found associated with the disease. Production of C3a and C5a in asthmatic lung may be due to the activation of the alternative complement pathway on allergen surfaces or by the proteases released by inflammatory cells that could cleave the C3 and C5. The anaphylatoxins, C3a and C5a, are known to cause leukocyte activation, smooth muscle contraction and vascular permeability. Humble et al (Nature, 2000. 406(6799): p. 998-1001), showed that a murine model of allergic airway disease with genetic deletion of the C3a receptor protects against the changes in lung physiology seen after allergen challenge. These results show the importance of complement C3a in disease pathology. Complement anaphylatoxins C3a and C5a have been identified as potential effectors in Type 1 hypersensitivity reactions (AHR), including urticaria, rhinitis and asthma. Thus, complement activation may synergize with classical IgE mediated responses, and inhibition of the complement pathway can prove therapeutic (Curr Opin Immunol, 2002. 14(6): p. 705-8).

In an ovalbumin (OVA)/Aspergillus fumigatus asthma model, C3-deficient mice exhibit strikingly diminished AHR and lung eosinophilia compared to wild type control mice. Furthermore, these C3-deficient mice had dramatically reduced numbers of IL-4 producing cells and attenuated antigen-specific IgE and IgG1 responses. Mice deficient in C3aR gene, demonstrated (Nature, 2000. 406(6799): p. 998-1001) near complete protection from the development of AHR to aerosolized methacholine. C3aR-deficient mice in the OVA/A fumigatus asthma model and demonstrated an attenuated allergic response very similar to C3-deficient animals with diminished AHR, eosinophil recruitment, TH2 cytokine production, and mucus secretion in the lung, as well as reduced Ag-specific IgE and IgG1 responses, thus, demonstrating a role for C3a in asthma. Abe and colleagues have reported evidence that links C5aR activation to airway inflammation, cytokine production and airway responsiveness. In their studies, inhibition of complement activation by soluble CR1, futhan (an inhibitor of complement activation) or synthetic hexapeptide C5a antagonist blocked the inflammatory response and airway responsiveness to methacholine. In studies using a blocking anti-C5 monoclonal antibody, Peng and colleagues found that C5 activation contributed substantially to both airway inflammation and AHR in the OVA model of asthma. Also, Baelder and colleagues reported that blockade of the C5aR substantially reduced AHR in the A. fumigatus model of asthma. These studies highlight the importance of C3a and C5a in the pathogenesis of experimental allergic asthma.

With respect to transplantation, complement activation significantly contributes to the inflammatory reaction following organ transplantation. In allotransplantation, the

complement system may be activated by ischemia/reperfusion while in xenotransplantation the major activators for complement are pre-existing antibodies. Animal model studies have shown that the use of complement inhibitors may significantly prolong graft survival. Thus, there is an established role of the complement system in organ injury after organ transplantation. Alternative pathway mediated activation appears to mediate renal ischemia/reperfusion injury, and proximal tubular cells may be both the source and the site of attack of complement components in this setting. Locally produced complement in the kidney also plays a role in the development of both cellular and antibody-mediated immune responses against the graft.

Hyperacute graft rejection (HAR) triggered by the activation of the recipient's complement system represents the major obstacle to successful xenotransplantation. After the binding of preformed antibodies to vascular glycoproteins, complement-induced activation and injury of endothelial cells with subsequent thrombosis leads to rapid destruction of foreign tissues. Inhibition of complement activation is therefore considered as a prerequisite for xenograft (Xg) survival. Both soluble CR1 and C1 inhibitors have shown benefit in the xenotransplatation (Zentralbl Chir, 1998. 123(7): p. 793-7).

In *in vivo* pig-to-primate heterotopic cardiac xenotransplantation model, porcine xenografts transplanted into untreated cynomolgus monkey recipients underwent HAR in 1 hr. A single intravenous bolus of sCR1 (15 mg/kg) administered to the recipient immediately before Xg reperfusion markedly inhibited total and alternative pathway serum complement activity and prolonged Xg survival to between 48 and 90 hr (n = 5)

(Transplantation, 1994. 57(3): p. 363-70). In a cardiac xenograft model, Davis et al showed that inhibition of complement and neutrophils were required to prolong xenograft survival (J Heart Lung Transplant, 1995. 14(5): p. 973-80). Kroshus et al demonstrated that anti-C5 monoclonal antibody inhibits cardiac injury in an *ex vivo* model of pig to human xenotransplantation. Further, it was found that anti-C5 MoAb prevents HAR (Proc Natl Acad Sci U S A, 1995. 92(19): p. 8955-9). These experiments further enlightened the role for complement in transplantation.

Activation of the complement system has also been implicated in the pathogenesis of a variety of central nervous system (CNS) or peripheral nervous system (PNS) diseases or injuries, including but not limited to multiple sclerosis (MS), myasthenia gravis (MG), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), Guillain Barre syndrome, reperfusion following stroke, degenerative discs, cerebral trauma, traumatic neuronal injury, Parkinson's disease (PD) and Alzheimer's disease (AD). It has now been shown that C3a and C5a receptors are found on neurons and show widespread distribution in distinct portions of the sensory, motor and limbic brain systems (Glia, 2002. 38(2): p. 169-73).

In brain trauma or hemorrhage, complement activation occurs and causes inflammation and edema within the brain. In a rat model of brain trauma, administration of sCR1 immediately prior to brain injury markedly inhibits neutrophil infiltration into the injured area, indicating complement was important for recruitment of phagocytic cells. Likewise, complement activation in patients following cerebral hemorrhage is clearly implicated by the presence of high levels of multiple complement activation products in both plasma and cerebrospinal fluid (CSF).

Multiple Sclerosis is characterized by a progressive loss of myelin ensheathing and insulating axons within the CNS. There is also clear evidence that complement plays a prominent role in the pathophysiology of CNS or PNS demyelinating diseases including MS, Guillain-Barre syndrome and Miller-Fisher syndrome

Complement contributes to tissue destruction, inflammation, clearance of myelin debris and even remyelination of axons. Studies have established that in an EAE mouse model, mice deficient in C3 or factor B showed attenuated demyelination as compared to EAE control mice. EAE mouse studies using a soluble form of a complement inhibitor coined "sCrry" and C3-/- and factor B-/- demonstrated that complement contributes to the development and progression of the disease model at several levels. In addition, the marked reduction in EAE severity in factor B-/- mice provides further evidence for the role of the alternative pathway of complement in EAE. In both multiple sclerosis and EAE, complement activation is thought to play a pivotal role by recruiting inflammatory cells, increasing myelin phagocytosis by macrophages, and exerting direct cytotoxic effects through the deposition of the membrane attack complex on oligodendrocytes. Mice that were deficient in either C3 or factor B clearly presented with reduced disease severity in this multiple sclerosis model. In both C3(-/-) and factor B(-/-) mice there was little infiltration of the parenchyma by macrophages and T cells. In addition, compared with their wild-type littermates, the CNS of both C3(-/-) and factor B(-/-) mice induced for EAE are protected from demyelination. These results suggest that complement is a target for the therapeutic treatment of inflammatory demyelinating diseases of the CNS (J Immunol, 2000. 165(10): p. 5867-73).

Additional evidence comes from the studies conducted on C6-deficient PVG/c rats. In the absence of C6 component, rats fail to synthesize MAC but not C3a and C5a. These rats exhibited no sign of demyelination when compared to normal controls. Neither demyelination nor axonal damage was seen in C6- rats. Reconstitution with C6 to C6 deficient rats yielded pathology and clinical disease similar to that seen in C6+ rats. In the absence of MAC deposition, complement activation leading to opsonization and generation of the anaphylatoxins C5a and C3a is insufficient to initiate demyelination (J Immunol, 2002. 168(1): p. 458-65).

Activation of complement is critically involved in both Guillain-Barré syndrome (GBS) and multiple sclerosis (MS). The effect of a complement inhibitor, soluble human complement receptor 1 (sCR1) blocks complement activation by both classical and alternative pathways (J Neuroimmunol, 1996. 67(1): p. 17-20) and demonstrates benefits in the disease.Myasthenia Gravis (MG) is an autoimmune disease of the neuromuscular junction in which autoantibodies against the acetycholine receptor target acetylcholine receptors resulting in loss of acetylcholine receptors and destruction of the end plate. sCR1 is very effective in ameliorating MG in an animal model of this disease, further indicating the role of complement in the disease.

Histological hallmarks of AD are senile plaques and neurofibrillary tangles. The senile plaques stain strongly for components of the complement system. Evidence points to a local neuroinflammatory state that results in neuronal death and cognitive dysfunction. Chronic localized inflammation is an important element of AD pathogenesis that causes significant neurodegenerative damage. The amyloid beta peptide has been implicated as a primary activator of complement in AD. The peptide

also localizes itself with drusen deposits categorizing them as sites of complement mediated inflammation. This suggests that amyloid deposition are an important component of the local inflammatory events that contribute to atrophy of the retinal pigmented epithelium, drusen biogenesis, and the pathogenesis of age related macular degeneration. The inflammatory deposits also contain C3 and fragments suggesting activation of complement (Exp Eye Res, 2001. 73(6): p. 887-96). Drusen deposits are also hallmark of glomerular nephritis and age related macular degeneration. Elevated levels of C3, C5 and C5b-9 (MAC) have been found associated with the drunsen deposits (Eye, 2001. 15(Pt 3): p. 390-5, Exp Eye Res, 2001. 73(6): p. 887-96).

In damaged regions in the brains of Parkinsons Disease (PD) patients, as in other CNS degenerative diseases, there is evidence of inflammation characterized by glial reaction (especially microglia), as well as increased expression of HLA-DR antigens, cytokines, and components of complement. These observations suggest that immune system mechanisms are involved in the pathogenesis of neuronal damage in PD. The cellular mechanisms of primary injury in PD have not been clarified, however, but it is likely that mitochondrial mutations, oxidative stress and apoptosis play a role. Furthermore, inflammation initiated by neuronal damage in the striatum and the substantial nigra in PD may aggravate the course of the disease. These observations suggest that treatment with complement inhibitory drugs may act to slow progression of PD.

Significantly higher C3b concentrations in patients with active dermatomyositis, Guillain-Barré syndrome and myasthenia gravis, compared to inclusion body myositis

and controls is suggestive of complement activation. (J Neuroimmunol, 1996. 71(1-2): p. 227-9) The *in-vitro* C3 uptake assay supports the role of C3b neoantigen and membranolytic attack complex deposition in the target tissues and may be a useful tool to monitor disease activity in patients with complement-mediated neurological disorders.

Complement activation has been implicated in the pathogenesis of a wide variety of chronic diseases; including rheumatoid arthritis (RA), juvenile rheumatoid arthritis, osteoarthritis, systemic lupus erythematosis (SLE), Behcet's syndrome and Sjogren's syndrome.

There are several publications documenting that complement activation products (C3a, C5a, and C5b-9) are elevated in the plasma of RA patients. Complement activation products have also been found within inflamed rheumatic joints. Positive correlations have been established between the degree of complement activation and the severity of RA. In both adult and juvenile rheumatoid arthritis, elevated serum and synovial fluid levels of Bb compared to C4d (a marker for classical pathway activation), clearly suggest that complement activation in RA is mediated predominantly via the alternative pathway. There is compelling evidence that immune-complex-triggered complement activation is a major pathological mechanism that contributes to tissue damage in rheumatoid arthritis (RA). Immune complex-mediated activation of complement through the classic pathway is believed to be one mechanism by which tissue injury occurs in RA patients. However, the amplification loop of the alternative pathway is required for classical pathway activation and propogation.

Animal models of experimental arthritis have been widely used to investigate the role of complement in the pathogenesis of RA. Intra-articular injection of the soluble form of complement receptor 1 (sCR1), a complement inhibitor, was found to suppress inflammation in a rat model of RA. Furthermore, sCR1 inhibits the development and progression of rat collagen-induced arthritis. Soluble CR1 inhibits the classical and alternative complement pathways at the steps of C3 and C5 activation in both the alternative pathway and the classical pathway, thereby inhibiting generation of C3a, C5a and sC5b-9. Studies using C5-deficient and sufficient strains indicate that C5 sufficiency is an absolute requirement for the development of CIA. Further evidence of the importance of C5 and complement in RA has been provided by the use of anti-C5 monoclonal antibodies (MoAbs). Prophylactic intraperitoneal administration of anti-C5 MoAbs in a murine model of CIA almost completely prevented disease onset while treatment during active arthritis resulted in both significant clinical benefit and milder histological disease.

Systemic lupus erythematosus (SLE) is an autoimmune disease of undefined etiology that results in production of autoantibodies, generation of circulating immune complexes, and episodic, uncontrolled activation of the complement system. Although the origins of autoimmunity in SLE remain elusive, considerable information is now available implicating complement activation as an important mechanism contributing to vascular injury in this disease. Activation of both the classical and alternative pathways of complement is involved in the disease. Both C4d and Bb are sensitive markers of moderate-to-severe lupus disease activity. Activation of the alternative complement pathway accompanies disease flares in SLE during pregnancy. In

addition, the lectin pathway may contribute to disease development since autoantibodies against MBL have recently been identified in sera from SLE patients.

Immune complex-mediated activation of complement through the classic pathway is believed to be one mechanism by which tissue injury occurs in SLE patients. Complement activation may be an important mechanism contributing to SLE pathogenesis. Results from animal models of SLE support the important role of complement activation in pathogenesis of the disease. Inhibiting the activation of C5 using a blocking anti-C5 MoAb decreased proteinuria and renal disease in NZB/NZW F1 mice, a mouse model of SLE. The alternative pathway also has an important role in the autoimmune disease manifestations of SLE since backcrossing of factor B-deficient mice onto the MRL/lpr model of SLE revealed that the lack of factor B lessened the vasculitis and glomerular disease. A humanized anti-C5 MoAb is under investigation as a potential treatment for SLE. This antibody prevents the cleavage of C5 to C5a and C5b. In Phase I clinical trials, no serious adverse effects were noted and more human trials are under way to determine its efficacy in SLE.

SLE is an example of systemic autoimmune diseases that affects multiple organs, including skin, kidneys, joints, serosal surfaces, and the CNS. SLE is frequently associated with severe vasculitis. Activated complement components appear in the circulation of patients with SLE. Elevated levels of C3a and C5a were identified in SLE patients. That these proinflammatory molecules are elevated during exacerbation of the disease suggest they may contribute to the vascular injury in SLE patients (Arthritis Rheum, 1986. 29(9): p. 1085-9, Arthritis Rheum, 1988. 31(5): p. 632-41). Horiquome et al showed that alternative complement pathway is activated in

membranoproliferative glomerulonephritis (MPGN) as suggested by the measurement of alternative pathway depenedent hemolysis of the patient plasma (Clin Exp Immunol, 1987. 70(2): p. 417-24). In a study using the cobra venom factor to deplete complement components, benefits in the disease were noticed in that glomerular deposits of complement C3b (C3c) deposits cleared within 24 hours of cessation of complement activation.

Results from both human and animal studies support the possibility that the complement system contributes directly to the pathogenesis of muscular dystrophy. Studies of human dystrophic biopsies have shown that C3 and C9 are deposited on both necrotic and non-necrotic fibers in dystrophic muscle. Using DNA microarray methods, Porter and colleagues found markedly enhanced gene expression of numerous complement-related mRNAs in dystrophin-deficient (mdx) mice coincident with development of the dystrophic disease.

Activation of the complement system has been implicated in the pathogenesis of a wide variety of renal diseases; including, mesangioproliferative glomerulonephritis (IgA-nephropathy, Berger's disease), membranous glomerulonephritis, membranoproliferative glomerulonephritis (mesangiocapillary glomerulonephritis), acute postinfectious glomerulonephritis (poststreptococcal glomerulonephritis), cryoglobulinemic glomerulonephritis, lupus nephritis, and Henoch-Schonlein purpura nephritis.

Kahn and Sinniah demonstrated increased deposition of C5b-9 in tubular basement membranes in biopsies taken from patients with various forms of glomerulonephritis. Another study of membranous nephropathy demonstrated a

positive correlation between increased sC5b-9 levels and poor prognosis. These various studies suggest that ongoing complement-mediated glomerulonephritis results in urinary excretion of complement proteins that correlate with the degree of tissue damage and disease prognosis.

Inhibition of complement activation in various animal models of glomerulonephritis has also demonstrated the importance of complement activation in the etiology of the disease. Couser et al. demonstrated the potential therapeutic efficacy of approaches to inhibit complement by using the recombinant sCR1 protein. Rats treated with sCR1 showed significantly diminished polymorphonuclear cell (PMN), platelet and macrophage influx, decreased mesangiolysis, and proteinuria versus control rats. Further evidence for the importance of complement activation in glomerulonephritis has been provided by the use of an anti-C5 MoAb in the NZB/W F1 mouse model. The anti-C5 MoAb inhibits cleavage of C5, thus blocking generation of C5a and C5b-9. Continuous therapy with anti-C5 MoAb for 6 months resulted in significant amelioration of the course of glomerulonephritis. A humanized anti-C5 MoAb (5G1.1) that prevents the cleavage of human complement component C5 into its pro-inflammatory components is under development by Alexion Pharmaceuticals, Inc., New Haven, Conn., as a potential treatment for glomerulonephritis.

Ulcerative colitis and Crohn's disease are chronic inflammatory disorders of the bowel that fall under the banner of inflammatory bowel disease (IBD). IBD is characterized by spontaneously occurring, chronic, relapsing inflammation of unknown origin. Despite extensive research into the disease in both humans and experimental animals, the precise mechanisms of pathology remain to be elucidated. However, the

complement system is believed to be activated in patients with IBD and is thought to play a role in disease pathogenesis.

It has been shown that C3b and other activated complement products are found at the luminal face of surface epithelial cells, as well as in the muscularis mucosa and submucosal blood vessels in IBD patients. Furthermore, polymorphonuclear cell infiltration, usually a result of C5a generation, characteristically is seen in the inflammatory bowel.

Complement activation plays a critical role in acute pancreatitis. In pancreatitis patients, significantly elevated levels of C3a, C5a and MAC were found compared to controls. As a result, down stream events such as increased vascular permeability, anemia and impaired respiration in these patients may be influenced by complement activation (Arch Surg, 1990. 125(7): p. 918-21). In additional studies, levels of C3a and C5a correlated to the severity of the disease (J Surg Res, 1989. 47(2): p. 138-43).

Studies by Gloor et al. has tested the presence of complement activation byproducts C3a and sC5b-9 and demonstrated that these were significantly elevated during the first 7 days in plasma of patients with severe acute pancreatitis, as compared to control patients. (Scand J Gastroenterol, 2003. 38(10): p. 1078-82) Pancreatic enzymes release proteases that cause activation of complement resulting in elevated levels of C3a and C5a. In the setting of animal model studies on experimental acute pancreatitis, the sera demonstrated excessive complement activation and neutrophil lung sequestration, an early event in acute pancreatitis. In sCR1 treated animals, the total complement activation was down regulated including neutrophil activation as indicated by the downregulation of CD11b expression.

(Surgery, 1997. 122(5): p. 909-17), Additionally, the underlying etiology of various skins diseases such as psoriasis support a role for immune and proinflammatory processes including the involvement of the complement system. Its activation leads to the generation of products that not only help to maintain normal host defenses, but also mediate inflammation and tissue injury. Proinflammatory products of complement include C3a, C4a, and C5a, and membrane attack complexes. Among them, C5a or its degradation product C5a des Arg, seems to be the most important mediator because it exerts a potent chemotactic effect on inflammatory cells. Intraderrnal administration of C5a anaphylatoxin induces skin changes quite similar to those observed in cutaneous hypersensitivity vasculitis that occurs through immune complex-mediated complement activation.

There is a significant activation of the alternative pathway following thermal injury. In thermal injury, chronic inflammation sets in if the injury remains untreated. The evidence for alternative pathway activation comes from the studies that determined the presence of elevated levels of complement activation byproducts in the plasma of burn patients. (Burns, 1998. 24(3): p. 241-4) Additional findings supporting a significant role for complement activation in thermal burn comes from studies using a rat thermal injury model in which a surface thermal burn led to lung injury. Treatment with anti-C5a antibodies were found to down regulate the C5a mediated damage (Shock, 1997. 8(2): p. 119-24).

The direct effect of four different radiographic contrast media (RCM) on the release of C3a and C5a and the production of IL-1 alpha and TNF-alpha from vascular endothelial cells was examined *in*, Activation of the complement system and cytokine

production by radiographic contrast media in vascular endothelial cells in vitro, Gyoten M.). The test RCM were as follows: diatrizoate (ionic monomer), iopamidol (nonionic monomer), ioxaglate (ionic dimer), and iotrolan (nonionic dimer). These were added to serum-free medium and adjusted to a final concentration of 1% (2.8 mg Iodine/ml). Human microvascular endothelial cells were stimulated by serum-free medium containing the test RCM for eight hours. After incubation, the media were aspirated and assayed for the concentrations of C3a, C5a, IL-1 alpha and TNF-alpha. Finally, the cells were harvested by trypsin, and their viability was determined by the dye-exclusion method. Diatrizoate and iotrolan had higher C3a release than the control ($p < 0.05$). No increase in C5a, IL-1 alpha or TNF-alpha levels was observed with any of the tested RCM, and there was no significant difference in cell viability with any of the tested RCM. The results of this study suggest that diatrizoate and iotrolan activated the complement system through the alternative pathway by directly stimulating vascular endothelial cells. These observations suggest that a direct effect of RCM on vascular endothelium might play a role in the pathogenesis of local drug eruptions due to RCM. X-ray contrast media that also activate complement. Eaton et al showed that commercial formulations of diatrizoate, iodamide, iothalamate, ioxaglate, iohexol, and

The Applicants respectively submit that the amount of experimentation needed by one having ordinary skill in the art to practice the claimed invention is not undue. The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation (MPEP 2164.01). Accordingly, the Applicant respectively submit that claims 32, 39, and 40 are enabled

because complement activation has been shown to be associated with the claimed diseases.

Claims 2-4 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and claim the subject matter. Claims 2-4 have been canceled. Therefore, withdrawal of the rejection of claims 2-4 is respectfully requested.

In view of the foregoing, it is respectfully submitted that the above-identified application is in condition for allowance, and allowance of the above-identified application is respectfully requested.

Please charge any deficiency or credit any overpayment in the fees for this amendment to our Deposit Account No. 20-0090.

Respectfully submitted,

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